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STUDIES ON THE BIOLUMINESCENCE OF THE MARINE OSTRACOD CRUSTACEAN *CYPRIDINA SERRATA*[1]

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At present, at least three species of marine ostracods belonging to the family Cypridinidae are known to be luminous. Among these, the luminescence of only one species has been well-studied. This is *Cypridina hilgendorfii* Müller, 1890, a species found in the coastal waters of Japan. Extensive studies carried out during the past 50 years have yielded detailed knowledge concerning the mechanism of bioluminescence. The organism lives in the sand and comes out to feed at night. When mechanically disturbed, the organism produces a blue luminescence by ejecting luciferin and luciferase into the surrounding sea water from two separate glands. The light-emitting reaction involves the oxidation of luciferin by molecular oxygen, catalyzed by the enzyme luciferase.

The second species is *Cypridina noctiluca* Kajiyama, 1912. In contrast to *C. hilgendorfii*, *C. noctiluca* is a free-swimming pelagic form. It is widely distributed along coastal waters in the western Pacific from southern Japan and Hawaii to Australia and Southeast Asia, and in the Indian Ocean. Haneda (1940) observed *C. noctiluca* at Palau Island. When the beam of an electric light was directed into the water where large numbers of the organisms were swimming, a bright luminous response was obtained. When a plus (+) mark was written on the surface of the water and the light immediately extinguished, a luminous plus (+) mark could be observed in the darkness. Other plankton organisms did not give the same response. Haneda (1953) also studied *C. noctiluca* at Hachijo Island, a subtropical island located approximately 200 kilometers south of Honshu, Japan. The secretory behavior and color of light of *C. noctiluca* were the same as *C. hilgendorfii*. Light resulted (positive luciferin-luciferase reaction) when a hot-water extract (luciferin) and a cold-water extract (luciferase) of the organism were mixed. These extracts also gave reciprocal light-emitting cross-reactions with hot- and cold-water extracts of *C. hilgendorfii*.

The third species is *Vargula harveyi*, recently reported from Jamaica, West Indies, by Seliger and McElroy (1965) and described by Kornicker and King (1965). Measurement of the bioluminescence emission spectrum showed a peak at 478 nm, close to the peak of 465 nm for *C. hilgendorfii*.

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The subject of this paper concerns a fourth species, *Cypridina serrata* Müller, 1906. *C. serrata* was collected during the recent R/V Alpha Helix Biological Expedition to New Guinea. Little is known about the distribution of this organism. The specimens studied by Müller (1906) were obtained from three locations: Paternoster Island (Station 40), approximately 118°E, 7°S in the Flores Sea, Indonesia; North Ubian (Station 99), 120°26′E, 6°7′N, Philippines; and Sulu Archipelago (Station 109), Philippines. The present finding of *C. serrata* at Madang, New Guinea, suggests that the organism is a widely distributed species in the western Pacific. We present herein the first observations of luminescence in this organism.

Materials and Methods

Cypridina serrata were collected in a cove in Kranket Island next to Dallman Passage in Madang Harbor, New Guinea. They were also found off Beliau Island and in Binnen Harbor, but not in such large numbers. *C. serrata* were located by shining a flashlight into the water in 2–3 second bursts. This caused elliptical clouds of bright blue luminescence, around 2–15 cm long, to appear in the water, presumably as a result of *C. serrata* organisms ejecting luciferin and luciferase into the sea water.

Collection was carried out by towing two weighted 38-mesh plankton nets (2–3 miles/hour) at a depth of about 1 meter below the surface through waters showing the best flashlight response and emptying them periodically into buckets containing sea water.

Towing distance from shore ranged from 2–15 meters along a shore length of approximately 1 kilometer. At high tide, the depth within this area varied between 2–10 meters. An attempt was made to collect *C. serrata* by lowering fish heads attached to strings in the water, the same method used for collecting *C. hilgendorfii* in Japan, but it was unsuccessful. All collections were made between 8–11 PM. Buckets containing the night's collection were strained first through a coarse wire mesh, then through a 74-mesh plankton net screen. When examined microscopically, the filtered material consisted largely of copepods, non-luminous shrimps, siphonophore fragments and *C. serrata*. Among these organisms, only *C. serrata* was found to luminesce spontaneously. Microscopic counts showed 2000–4000 *C. serrata* in a single night's collection. Each such collection was washed with clean sea water, then resuspended in a small volume of sea water and filtered through Whatman #2 folded filter paper. The filtered residue was then either freeze-dried or used directly in preparing luciferase. In the latter case, the entire batch was ground with a mortar and pestle, washed with about 40 ml of distilled water into a dialysis bag and dialyzed for 60 hours in an ice box against 4 changes of distilled water. The contents of the bag were then centrifuged for 30 minutes at 1500 × *g* in an Aloe conical centrifuge. The supernatant was decanted, the precipitate washed twice with 10 ml of distilled water and centrifuged. The supernatants were combined and dialyzed for 48 hours in the refrigerator against 4 changes of distilled water. The contents of the bag were freeze-dried, redissolved in 12 ml of 0.07 *M* sodium phosphate buffer, pH 6.8, dialyzed 16 hours against the same buffer, and put on a 45 cm Sephadex G-200 column equilibrated against

the buffer. The column was eluted with the buffer and 0.02 ml of each collected fraction (fraction volume, 6.3 ml) was assayed for luciferase activity by mixing with 2.0 ml of 0.2 *M* sodium phosphate buffer, pH 6.8, and injecting into a vial containing 0.05 ml of *C. hilgendorfii* luciferin. Light intensity was measured in arbitrary light units using a photomultiplier photometer. The active fractions were combined, dialyzed 60 hours against many changes of distilled water, and freeze-dried. Further purification of luciferase was not carried out due to limited quantities of the organism.

C. serrata luciferin used for kinetic and oxygen-requirement experiments was prepared by grinding 0.4 g of the freeze-dried material in 15 ml of boiling distilled water in an all-glass homogenizer for 1 minute. The suspension was then quickly cooled in an ice bath while bubbling with 99.99% argon. After centrifuging at 12,000 × *g* at 4° C for 8 minutes, the clear straw-colored solution of luciferin supernatant was stored under argon in an ice-bath until used. Luciferin used in the chromatographic experiments was prepared separately, by extracting 0.25 g of powdered organisms in 15 ml of absolute methanol for 5 hours in an argon atmosphere. After centrifuging at 12,000 × *g* at 4° C for 10 minutes, 3 ml of the clear brown supernatant were evacuated to dryness, redissolved in a few drops of absolute methanol, and spotted directly on the Whatman filter paper.

C. hilgendorfii luciferase was prepared in a highly purified form by the method of Tsuji and Sowinski (1961), and in a partially purified form by the same method as for *C. serrata* luciferase for comparative purposes.

C. hilgendorfii luciferin was purified by the method of Tsuji (1955), except that the initial extraction of luciferin from the defatted *Cypridina* powder was carried out with absolute methanol instead of butanol. Nine ml of the final ice-cold butanol solution of luciferin were evacuated to dryness, redissolved in 30 ml of 0.1 *N* HCl, and used directly. For chromatographic experiments, some of the dry luciferin was redissolved in a few drops of absolute methanol, and used directly.

Chromatography of *C. serrata* and *C. hilgendorfii* luciferins was carried out according to the method of Tsuji (1955), using Whatman No. 3MM paper and a solvent mixture of ethyl acetate, ethyl alcohol, and water (5:2:3 by volume). Chromatography was carried out at 23° C for 1 hour and 15 minutes.

Antibody to *C. hilgendorfii* luciferase was prepared as purified gamma globulin fraction from pooled antisera of rabbits immunized against luciferase as described by Tsuji *et al.* (1969).

Light intensity was measured using a photomultiplier photometer and a chart recorder.

Results

1. *General observations*

Cypridina serrata organisms are shown in Figure 1. Two other forms, *Cypridina inermis* Müller, 1906, and *Metavargula* species, of undetermined luminosity, were also collected in small numbers in the same area but were not studied. The specimens were identified (which included dissection studies) by Dr. Louis S. Kornicker of the Smithsonian Institution. Specimens of all three species are on deposit at the U. S. National Museum. The depository numbers

and size of two of the specimens of *C. serrata* (dissected) are as follows: 128152, male, length 1.66 mm, height 0.86 mm; 128153, female, length 1.53 mm, height 0.86 mm.

The bright bluish luminous clouds, produced by *C. serrata* when stimulated with a flashlight (see *Discussion* section), were beautiful and impressive, and probably represent a unique display among luminous organisms. Short 1 second bursts of light produced relatively few clouds and 2 second bursts produced the maximum number of clouds, approximately 50–100 per cubic meter of water. Spontaneously produced luminous clouds were not observed in the water. The response to a

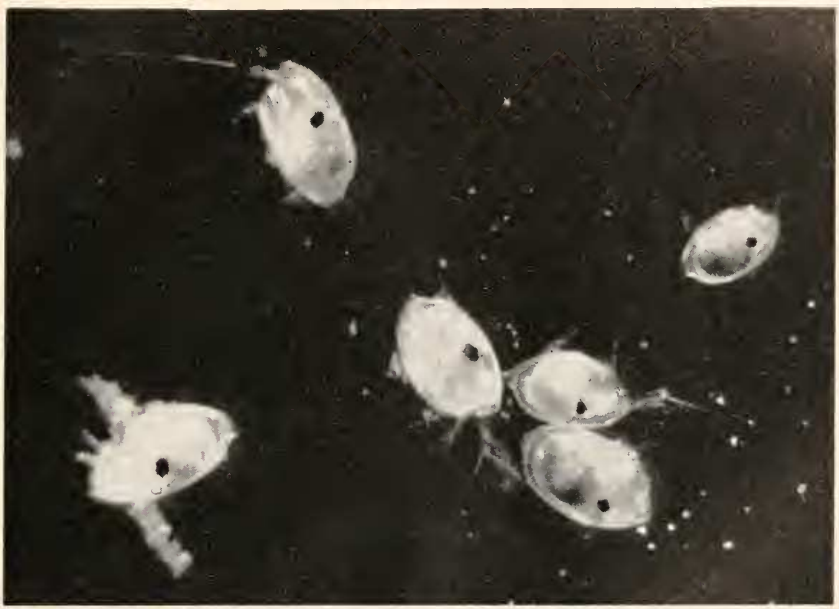

FIGURE 1. *Cypridina serrata,* actual size about 1.6 mm; Madang Harbor, October, 1969.

single stimulation was strikingly uniform: the luminous clouds burst instantly and decayed within 3–4 seconds after the flashlight was turned off. A given water volume usually responded 3–5 times to the flashlight before becoming refractory to further stimulation; that is, shining light into the water did not elicit any more luminous clouds. The interval between flashlight bursts was 4–5 seconds. A refractory water volume did not respond to further flashlight stimulation, but after remaining in darkness for 20 minutes a few luminous clouds could be produced.

Many of the luminous clouds were located 1 meter or more below the surface of the water. Often when these clouds were scooped up with a plankton dip net, a bright blue luminous spot was detected on the net and from it a copious luminous secretion soon began to flow down the side of the net. When the organism was touched or gently teased off the net with a finger for identification, the finger became intensely streaked. These large bright blue spots, often continuously luminous, are to be contrasted with the myriads of tiny flashes of light

that are seen when the net is first raised. The light in the latter instance is primarily due to copepods and siphonophores and soon dies down leaving only the bright blue luminous spots of *C. serrata*. The color of light was similar, if not identical, to that of living *C. hilgendorfii*. When the plankton tow nets were emptied several times into the bucket, the sea water in the bucket turned brightly luminous. Such sea water, when passed through filter paper, produced light when mixed with *C. hilgendorfii* luciferin. Slight mechanical disturbance of the water in the bucket readily caused *C. serrata* organisms to emit a brilliant blue glow, lighting up the bucket for many seconds. When the water in the bucket was swirled, long trails of blue luminous secretion could be observed.

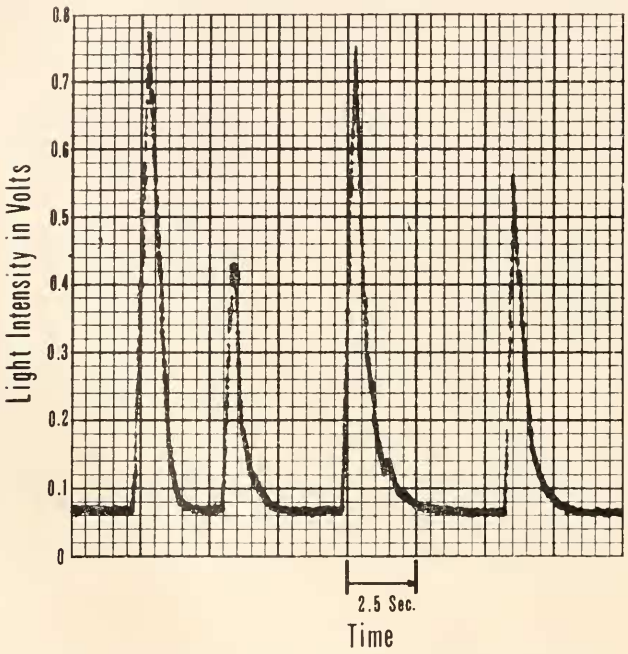

FIGURE 2. Apparent spontaneous flash recordings of 3 *Cypridina serrata* organisms in sea water, recorded with a photomultiplier photometer and strip-chart recorder (Mini-Writer, Watanabe Inst. Co.).

Light response to stimulation was less on moonlit nights. On one moonlit night about 15 individual responses were counted in a 1 hour period, whereas earlier in the month when the moon was dark, thousands of responses could be counted in the same area. On such moonlit nights, several hundred *C. serrata* could still be collected in an evening by towing plankton nets. These organisms were apparently refractory to light stimulation since only a few responses were obtained in the collection area with a flashlight. These organisms also gave almost no streaking in the collecting net and few spontaneous flashes.

2. Flash recordings

Collected *C. serrata* did not respond to stimulation by flashlight immediately after collection or later in the laboratory. However, they did emit apparent spontaneous flashes of light which were recorded. A typical set of four flashes is shown in Figure 2. This was made by placing 2 or 4 organisms in a vial containing 10 ml of sea water and monitoring the vial in a photometer. The organisms were isolated with a small glass capillary from a mixture of organisms in a Petri dish with the aid of a low-power microscope. Visual inspection of a vial of *C. serrata*

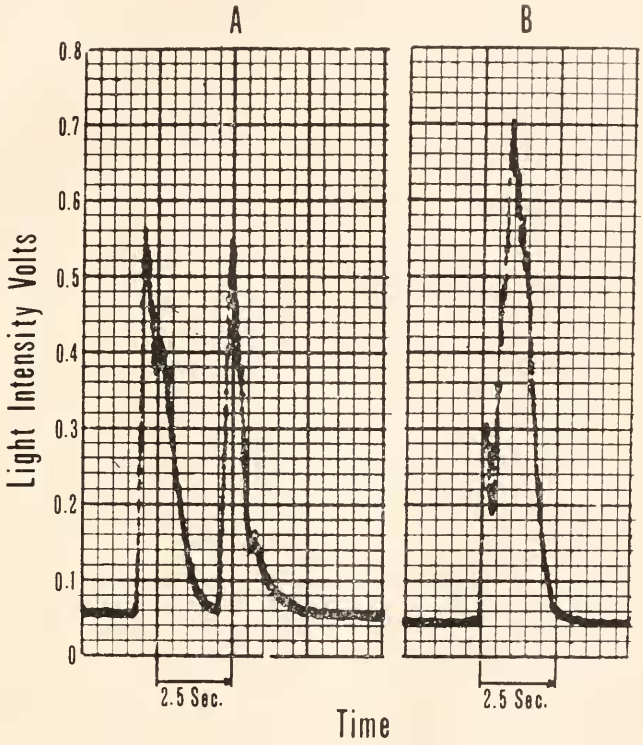

FIGURE 3. Apparent spontaneous flash recordings of 3 *Cypridina serrata* organisms in sea water showing (A) double flash and (B) possibly triple flash (Mini-Writer, Watanabe Inst. Co.).

organisms showed the flashes to be either point sources of light, or in some cases, long thread-like streaks or jets of luminous secretion which issued from the organisms. A point source of light was about the size of the body of *C. serrata* so that the whole organism appeared luminous. The light seemed to be of internal origin and did not show any sign of luminous secretion diffusing into the water. Some of the apparent spontaneous flashes were extremely bright, easily visible to the naked eye in a lighted laboratory room whereas some point sources of light were glows that lasted for many minutes (some were watched for over 30 minutes). The flashes in Figure 2 each show a duration of approximately 1.5 seconds. The

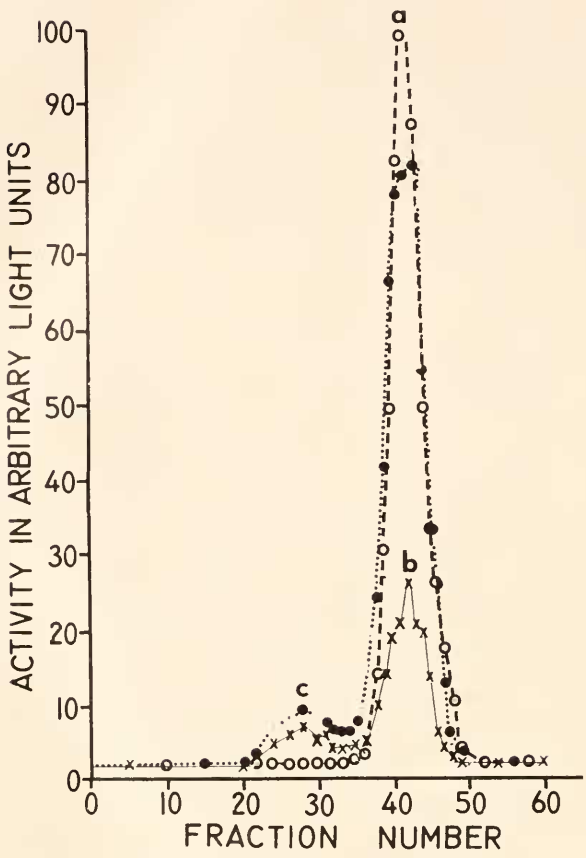

FIGURE 4. Elution patterns of *Cypridina serrata* luciferase (Curve *a*), *Cypridina hilgendorfii* luciferase (Curve *b*), and mixture of *C. serrata* and *C. hilgendorfii* luciferases (Curve *c*) from Sephadex G-200 column. (Note: overlap of some points.)

shape of the flash curve usually varied from those shown in Figure 2 to some with a double spike. The frequency of flashing was irregular and varied with each batch of organisms. The number of active batches, however, was relatively small. Organisms that did not start flashing within 10 minutes after being placed in the photometer usually did not flash later.

A plot of the logarithm of light intensity (Fig. 2) against time shows that the decay for each curve is exponential, with rate constants, from left to right, of 1.43 sec^{-1}; 0.863 sec^{-1}; pre-spike, 1.13 sec^{-1} and post-spike, 0.818 sec^{-1}; and 0.946 sec^{-1}. In the third curve from the left, the exponential decay is interrupted by an after-spike, but the after-spike also decays exponentially. The after-spike appears to be a second flash superimposed on the first. It may be due to triggering in view of the known response of the organism to artificial light. Fig. 3A shows two additional flash recordings with double spikes. Assuming that one animal triggers a second animal, the estimated latency was 500–800 milliseconds for the flash response.

In Figure 3B, which represents still another flash recording, the curve appears to be a superimposition of 3 flashes.

3. *Chromatography of luciferase*

Twelve milliliters of partially purified *C. serrata* luciferase solution possessing a concentration of 0.40 mg/ml and 12 ml of partially purified *C. hilgendorfii* luciferase solution possessing a concentration of 0.25 mg/ml were prepared in

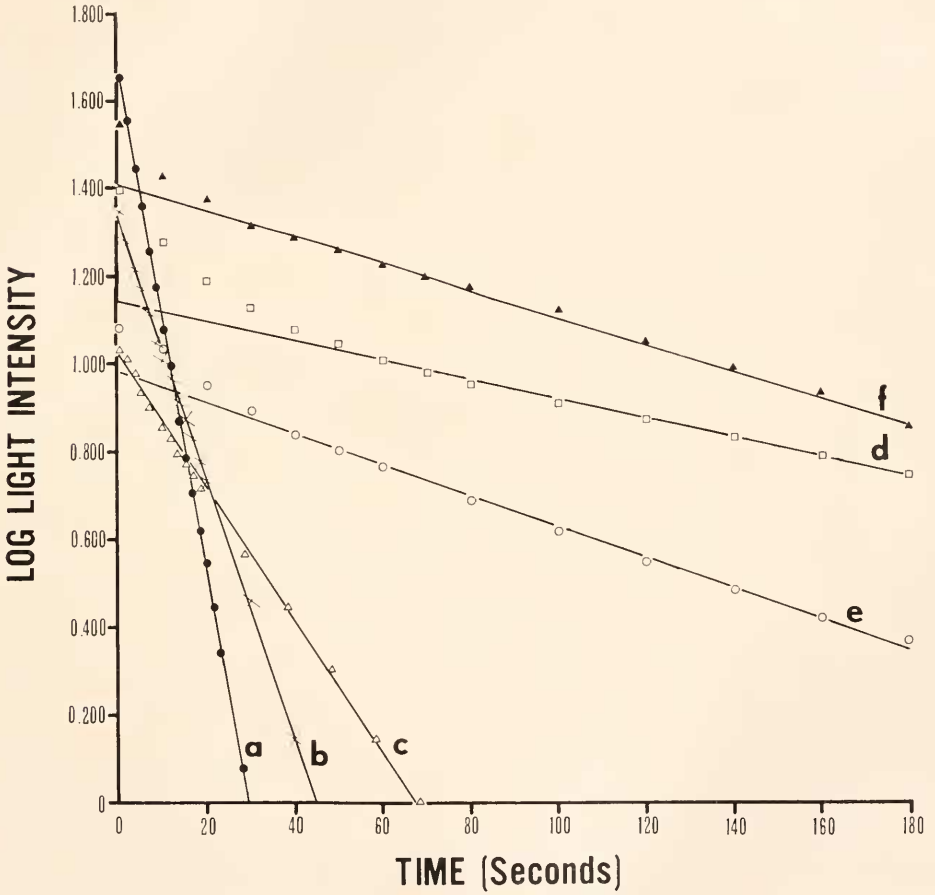

FIGURE 5. Luminescence intensity decay curves of *Cypridina serrata* luciferin-luciferase mixtures, except as noted. For each decay measurement, 1.0 ml of a solution of luciferase dissolved in 0.1 *M* sodium phosphate buffer, pH 6.8, was injected with a hypodermic syringe into a mixture of 1.5 ml of luciferin + 1.5 ml of 0.1 *M* sodium phosphate buffer, pH 6.8, except for curve *e*, in which luciferase was injected into 0.75 ml luciferin + 2.25 ml buffer. Luciferase concentrations: curve *a*, 2.0 mg/ml; curve *b*, 1.0 mg/ml; curve *c*, 0.5 mg/ml; and curves *d* and *e*, 0.133 mg/ml. Curve *f*: 1.0 ml *C. hilgendorfii* luciferase (1.54×10^{-4} mg/ml) injected into 1.5 ml luciferin + 1.5 ml buffer. Curve *e* is shifted upward 0.2 logarithm units for better comparison. Two separate preparations of *C. serrata* luciferin were used: one for curves *a*, *b*, and *c*, and another for curves *d*, *e*, and *f* (Bristol Recorder).

0.07 *M* sodium phosphate buffer, pH 6.8. On assay with *C. hilgendorfii* luciferin, the *C. serrata* luciferase solution possessed a somewhat higher activity than the *C. hilgendorfii* luciferase solution. Four milliliters of each of these preparations were mixed together, the 8 ml remainder of the luciferase solutions being frozen. The 8 ml of mixture were dialyzed 16 hours against the same buffer and put on a 90 cm Sephadex G-200 column. Each eluted fraction (6.3 ml each) was assayed with *C. hilgendorfii* luciferin. One day later the 8 ml of *C. serrata* luciferase solution were thawed, dialyzed as above, and put on the same column. Still another day later, the *C. hilgendorfii* luciferase solution was thawed, dialyzed as above, and put on the same column. The elution curves are shown in Figure 4. All three solutions showed a single activity peak, which appeared at the same place (fraction 42) in the elution diagram. Hold-up volume, measured with 2% dextran blue solution, was 135 ml. Both solutions containing *C. hilgendorfii* luciferase showed small peaks at the beginning of elution due to what might be a polymerized form of the luciferase. We conclude that *C. serrata* and *C. hilgendorfii* luciferases are indistinguishable by gel elution chromatography.

4. *Kinetic measurements*

The decay of light intensity in various mixtures of *C. serrata* luciferin and luciferase was measured. The results are shown in Figure 5, with logarithm of light intensity, which is a measure of reaction rate, plotted against time. In the first experiment, represented by curves *a*, *b*, and *c*, the concentration of luciferin was held constant and the luciferase concentration was varied so as to give concentrations of C, C/2, and C/4, respectively. In each case, the decay of luminescence followed first order kinetics. The calculated rate constants were 5.67×10^{-2} sec^{-1}, 3.07×10^{-2} sec^{-1}, and 1.52×10^{-2} sec^{-1}, respectively. The corresponding half-times for the decays were 5, 10, and 20 seconds. The rate constants are, therefore, observed to be directly proportional to luciferase concentration. A second experiment, represented by curves *d* and *e*, was run at a lower luciferase concentration. Luciferase concentration was the same (0.133 mg/ml) but the luciferin concentration of *e* was one-half that of *d*. The luminescent reaction initially showed a very high rate of decay, but it soon became first order. The rate constants for *d* and *e* were 1.97×10^{-3} sec^{-1} and 3.34×10^{-3} sec^{-1}, respectively. The rate was thus increased by a factor of 1.70 (rate constant *e*/rate constant *d*) on a one-half decrease in luciferin concentration. The rate constant therefore appears to be dependent on luciferin concentration. Because of a limited supply of *C. serrata* organisms, the experiment could not be carried out with purified *C. serrata* luciferin. However, when *C. serrata* luciferin was replaced with highly purified *C. hilgendorfii* luciferin, the first order rate constants were found to be directly proportional to luciferase concentration and completely independent of luciferin concentration. In the final experiment, *C. serrata* luciferin was run against a single concentration of *C. hilgendorfii* luciferase (1.54×10^{-4} mg/ml). The decay curve, *f*, was typical of the curves previously obtained with a rate constant of 3.00×10^{-3} sec^{-1}. Immediately after mixing, a high initial rate of decay occurred which was followed quickly by a normal first order decay.

5. *Oxygen requirement*

Requirement for oxygen in the luminescent reaction was demonstrated by placing 3.0 ml of *C. serrata* luciferin diluted with 8.0 ml of 0.1 *M* sodium phosphate buffer, pH 6.8, in one arm of a mixing apparatus, and 5.0 ml of *C. serrata* luciferase solution in a second arm. Argon (99.99%) was bubbled through both arms for 12 minutes, then the apparatus was evacuated for 2 minutes with a vacuum pump. The arm containing the luciferin was placed in the cell holder of a photomultiplier photometer, and the luciferase was then added under vacuum. No

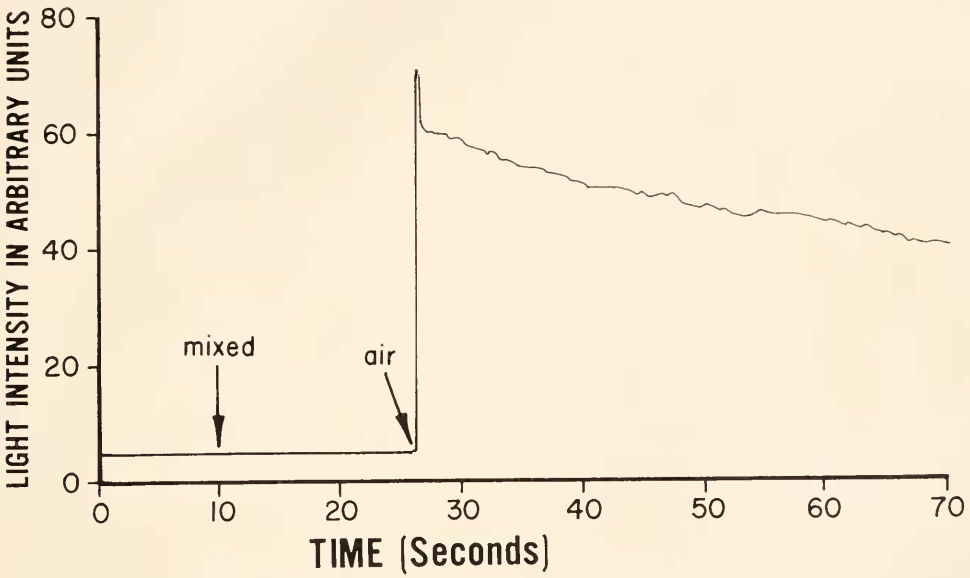

FIGURE 6. Light intensity measurement (tracing of recording) showing requirement for oxygen. *Cypridina serrata* luciferase and luciferin were mixed in absence of oxygen, then air introduced 17 seconds later. Note the high initial rate of decay (Leeds and Northrup Speedomax Recorder).

light was observed. After 17 seconds, air was admitted into the mixture through a capillary tube. The results are shown in Figure 6. The admission of air produced a bright initial burst of light. The high initial rate of decay was rapidly followed by a normal first order decay. The experiment was repeated with *C. hilgendorfii* luciferin and luciferase with the same result. The data indicate that oxygen is required by *C. serrata* in the luminescent reaction.

6. *Inhibition by antibody*

The immunological inhibition experiments are summarized in Table I. Rabbit antibody to *C. hilgendorfii* luciferase was incubated separately with *C. hilgendorfii* and *C. serrata* luciferases, closely matched in activity. After incubation, the residual luciferase activity remaining was determined in the separate mixtures. The antibody is seen to inhibit *C. hilgendorfii* luciferase to a far greater extent

than *C. serrata* luciferase. We conclude that *C. serrata* luciferase, while possessing similar catalytic activity, is immunochemically different from *C. hilgendorfii* luciferase.

7. *Chromatography of luciferin*

Paper chromatography of *C. serrata* and *C. hilgendorfii* luciferins gave identical R_f values (average of 0.65). We conclude that the luciferins are very similar, if not identical.

Discussion

According to Harvey (1952), the genus *Cypridina* includes between 20 and 25 species. Among these, two species, *Cypridina hilgendorfii* and *C. noctiluca*, are known to be luminous. The luminescence of *C. hilgendorfii* has been extensively

Table 1

Inhibition of luciferase activity by rabbit antibody

Incubation mixture	*Cypridina hilgendorfii*		*Cypridina serrata*	
	Initial light intensity in arbitrary units	Per cent of control	Initial light intensity in arbitrary units	Per cent of control
Control	42.0†	100	44.5‡	100
Control + 2.0×10^{-2}mg antibody*	24.0	57	41.0	92
Control + 6.0×10^{-2}mg antibody*	7.5	18	28.5	64
Control + 18.0×10^{-2}mg antibody*	2.0	5	15.0	34

* Prepared from pooled antisera of rabbits immunized against *C. hilgendorfii* luciferase.

† Control consisted of 1.54×10^{-4} mg of purified *C. hilgendorfii* luciferase dissolved in 1.0 ml of 0.1 *M* sodium phosphate buffer, pH 6.8.

‡ Control consisted of 1.33×10^{-1} mg of partially purified *C. serrata* luciferase dissolved in 1.0 ml of 0.1 *M* sodium phosphate buffer, pH 6.8. Note: All mixtures were brought up to 1.5 ml final volume with 0.1 *M* sodium phosphate buffer, pH 6.8, after adding antibody. After 19 hours incubation at 4° C, luciferase activity in a 1.0 ml aliquot was determined as the initial maximal light intensity observed on adding a constant saturating concentration of *C. hilgendorfii* luciferin

studied and needs little comment. *C. noctiluca* has been studied on different occasions by Haneda (1940, 1953, 1955). In addition, *Cypridina norwegica* is reported by Harvey (1952) to yield extracts that give light-emitting cross-reactions with extracts of *C. hilgendorfii*. Two other positive luciferin-luciferase cross-reactions have been reported. A close relation, *Pyrocypris*, a genus belonging to the family Cypridinidae, has been shown by Harvey (1922) to give a luciferin-luciferase cross-reaction with *C. hilgendorfii*. A *Cypridina* species from Jamaica, which emits a bluish yellow or yellowish light, has been found by Harvey (1924) to cross-react with *C. hilgendorfii*, which emits a bluish light. Harvey found that *C. hilgendorfii* luciferase when cross-reacted with Jamaican *Cypridina* luciferin gave a bluish light, whereas Jamaican *Cypridina* luciferase when cross-reacted with *C. hilgendorfii* luciferin gave a yellowish light. The results indicated that the color of light depended on the source of luciferase. More recently, a new

luminous species, *Vargula harveyi*, has been reported from Long Bay, northeast Jamaica, by Seliger and McElroy (1965) and described by Kornicker and King (1965).

The present study illustrates the difficulty that may be encountered in trying to identify an organism that luminesces at sea unless specimens are taken. The evidence in the present instance is not unequivocal, but several reasons suggest that the organisms involved at Madang are *Cypridina serrata*. First, there was a direct correlation between the flashlight response and the presence of *C. serrata* in the water. *C. serrata* was absent from areas where the flashlight response was negative. When the plankton net was towed in such areas, the net brought up only mixtures of copepods, non-luminous shrimps, siphonophores and some dinoflagellates. No bright blue luminous spots or *C. serrata* organisms were found in the net. Second, the blue color of the luminous cloud was similar to the color of light produced by *C. serrata* in the laboratory. This evidence, however, cannot be considered very strong since the color of luminescence of most marine organisms is bluish. Third, the cloud was observed to drift in the water during the 3–4 seconds of decay. The size of the cloud was many times greater than any of the luminous organisms collected in the water. These observations are difficult to explain except under conditions in which luminous substances or components that react to produce light are ejected into sea water and afterwards diffuse or are carried by the current. Fourth, the behavior of these organisms toward moonlight was similar to *C. hilgendorfii*. On moonlit nights, few organisms were found to respond to light stimulation and only a relatively small number appeared in the water. In Japan, the authors have also observed that *C. hilgendorfii* organisms appear in smaller numbers on moonlit nights.

Another question which naturally arises concerns the origin of luciferin and luciferase in the extracts studied. The freeze-dried material contained both *C. serrata* and a mixture of luminous and non-luminous copepods, two closely related ostracods. The luciferins and luciferases from these organisms could possibly give light-emitting cross-reactions with each other and with the luciferin and luciferase of *C. hilgendorfii*. However, careful studies conducted by Harvey (1926) seem to rule out this possibility. Harvey showed that the hot- and cold-water extracts of copepods do not give the luciferin-luciferase reaction with each other and do not cross-react with the extracts of *Cypridina*. Thus, the luciferin and luciferase in this study appear to be of *C. serrata* origin.

The response of *C. serrata* to light stimulation is strikingly similar to that of *C. noctiluca*, observed by Haneda (1940). The mechanism whereby a luminous cloud is produced by *C. serrata* is undoubtedly similar to that in *C. hilgendorfii*. In the latter, luciferin and luciferase are ejected into the water from storage glands, where on diffusion, the light reaction takes place. The elucidation of the light-sensitive response mechanism awaits future study. The finding of the luminescence response system in *C. serrata* may be considered significant since light-stimulated luminescence is unknown except for *C. noctiluca* and fireflies exposed to species-specific flash patterns. Turner (1966), however, states that numerous reports by mariners exist in which luminescence has been observed when light is shone into the sea.

C. serrata is stimulated to luminesce either mechanically or by artificial light and, in addition, luminesces spontaneously; but *C. hilgendorfii* is known to be stimulated only mechanically. Although *C. serrata* did not respond to flashlight stimulation when once placed in the collection bucket, it did respond when the water was agitated with the hands. The non-responsiveness of captive *C. serrata* to flashlight stimulation was not further investigated and, therefore, no explanation can be given for the lack of response.

The flash recordings of isolated *C. serrata* organisms in Figures 2 and 3 suggest that the flash of one organism (produced either spontaneously or by collision with the wall or another organism) triggers a neighboring organism to flash. This assumption is reasonable because of the known response of *C. serrata* to artificial light and by the fact that a flash pattern containing an after-spike is not normally observed with luminous organisms. No comparisons can be made between the *C. serrata* flash and the *C. hilgendorfii* flash, as no records exist, but the dinoflagellate *Noctiluca* flash has been investigated by Nicol (1958), Hastings (1959), and Eckert (1965, 1967). These results show a flash duration of about 100 milliseconds, a latency of 2–5 milliseconds, and a mean rate constant for the exponential decay of luminescence of 0.088 milliseconds^{-1}. Compared to the *C. serrata* flash, the flash duration is about 15 times shorter, the latency is about 200 times shorter, and the decay rate is approximately 100 times faster. Data on the copepod flash, however, are not available in the literature and no comparison can be made. Since the duration of a flash depends on factors such as mixing and concentration of reactants, it would be difficult to compare a recorded flash with a light-stimulated flash in the ocean, and with light emission from a mixture of luciferin and luciferase.

The luminous cloud, the thread of luminous secretion, and the point source of light, all produced by *C. serrata*, indicate that the organism is able to control luminescence. It is possible that the quick ejection of a cloud of luminous material serves to propel the organism through the water and provide a screen to escape from a predator. The production of a point source of light appears to indicate a mixing of luciferin and luciferase within the organism or mixing just at the gland orifices through a finely regulated release of luciferin and luciferase. The ability of *C. serrata* to control its luminescence would account for the luminous clouds observed in the sea and the flashes observed in the laboratory. When collecting *Vargula harveyi* at Long Bay, Jamaica, in 1965 and 1967, Dr. Howard Seliger (personal communication) of the McCollum-Pratt Institute, Johns Hopkins University, observed point source of luminescence similar to ours. The whole body of *V. harveyi* was luminous without any appearance of luminous secretion and he believes that the mixing of luciferin and luciferase takes place by one of the above two mechanisms.

In the reaction between crude *C. serrata* luciferin and partially purified luciferase, the decay rate is initially very high. This anomaly was first observed by Amberson (1922) in the *C. hilgendorfii* luciferin-luciferase reaction. In his early experiments, mixing *C. hilgendorfii* luciferin and luciferase resulted in a bright initial flash of light, corresponding to a high initial rate of decay, which was then followed by a normal first order decay. The bright initial flash was

attributed by Amberson (1922) to the active site of the enzyme being free of luciferin at the outset.

If *C. serrata* luminescence is due to a first order reaction, theoretically the rate constant should be independent of luciferin concentration. However, a near doubling of the rate constant occurs when the luciferin concentration is halved (Fig. 5d and e), indicating that the rate constant is dependent on luciferin concentration. The rate constant, however, is independent of luciferin concentration when highly purified *C. hilgendorfii* luciferin is used. A similar result has also been noted in the *C. hilgendorfii* luminescent reaction. Amberson (1922), Stevens (1927), Harvey and Snell (1931), and Chase (1956) observed that the first order rate constant increased as the initial luciferin concentration was decreased. The rate constant increase was about two-fold in the studies of Amberson (1922) and Chase (1956) and five-fold in the experiments of Harvey and Snell (1931). Subsequently, Chase and Harvey (1942) found that the rate constant showed no change with luciferin concentration if highly purified luciferin and partially purified luciferase were used. They concluded that some impurity in either luciferin or luciferase was responsible for the increase in the rate constants of the earlier workers. In the present work with *C. serrata*, the result obtained by using purified *C. hilgendorfii* luciferin appears to indicate that the increase in the rate constant is due to an impurity in the *C. serrata* luciferin.

Three other aspects of the study require brief comments. The data (Fig. 6) show clearly that the luminescent reaction of *C. serrata* requires oxygen. The same requirement for oxygen was demonstrated many years ago for the *C. hilgendorfii* reaction by Harvey (1917, 1920). The immunochemical results (Table I) show that *C. serrata* luciferase is a related but distinct enzyme from *C. hilgendorfii* luciferase. Tsuji and Haneda (1966) have previously shown that antibody to *C. hilgendorfii* luciferase may be used to distinguish *C. hilgendorfii* luciferase from another closely related luciferase. Finally, the identical R_f's show that *C. serrata* and *C. hilgendorfii* luciferins are very similar, if not the same.

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Summary

1. The physical appearance and bioluminescence behavior, and light-emitting reaction of the marine ostracod crustacean, *Cypridina serrata*, are described.

2. In the natural environment of the sea, the free-swimming *C. serrata* appears to emit almost instantaneously a bright blue luminous cloud when stimulated with artificial light.

3. The method of light production, consisting of the ejection of luciferin and luciferase into sea water, and the color of light are similar to that of *C. hilgendorfii.*

4. In captivity, *C. serrata* emits apparent spontaneous flashes of light, whose duration is approximately 1.5 seconds, with an apparent latency of 500–800 milliseconds.

5. *C. serrata* luciferase cannot be distinguished from *C. hilgendorfii* luciferase by gel elution chromatography but may be distinguished immunochemically.

6. The luminescence of *C. serrata* is due to a first order reaction, similar to that of *C. hilgendorfii.* The luciferins and luciferases of both organisms cross-react to give light.

7. The luminescence of *C. serrata,* like *C. hilgendorfii,* is oxygen dependent.

8. *C. serrata* luciferin is similar, if not identical, to *C. hilgendorfii* luciferin when compared by paper chromatography.

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